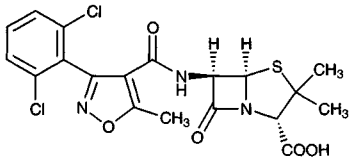
Dicloxacillin



Molecular formula: $C_{19}H_{17}Cl_2N_3O_5S$
Molecular weight: 470.33
CAS Registry No.: 3116-76-5, 13412-64-1 (sodium salt monohydrate), 343-55-5 (sodium salt)
Merck Index: 3134
Lednicer No.: 1 413

SAMPLE

Matrix: blood
Sample preparation: 400 µL Serum + 400 µL MeCN, vortex for 10 s, shake slowly for 15 min, centrifuge at 3000 g for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex for 10 s, shake for 15 min, centrifuge at 3000 g for 10 min, inject a 50 µL aliquot of the upper aqueous layer.

HPLC VARIABLES

Column: µBondapak C18
Mobile phase: MeCN:water:200 mM ammonium acetate 28:62:10, pH 5.6
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Retention time: 11.7
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Extracted: cloxacillin, methicillin, nafcillin, oxacillin
Noninterfering: amdinocillin (mecillinam), amikacin, amoxicillin, ampicillin, carbenicillin, cefamandole, cefazolin, ceforanide, cefatoxamine, cefoxitin, cephalexin, cephaloridine, cephalothin, cephradine, cepharin, chloramphenicol, clindamycin, co-trimoxazole, fluorocytosine, gentamicin, metronidazole, moxalactam, penicillin, piperacillin, sulfamethoxazole, theophylline, ticarcillin, tobramycin, trimethoprim, vancomycin

KEY WORDS

serum

REFERENCE

Rudrik,J.T.; Bawdon,R.E. Determination of penicillinase-resistant penicillins in serum using high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1981**, *4*, 1525–1545.

SAMPLE

Matrix: blood
Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 mL MeCN and 1 mL 10 mM pH 2 Na_2HPO_4. 250 µL Plasma + 100 µL water, add 400 µL MeCN at -15° while vortexing, add 700 µL 10 mM pH 2 Na_2HPO_4, centrifuge at 8000 g for 10 min. Add the supernatant to the SPE cartridge, wash with 1 mL water, elute with two 500 µL portions of MeCN:water 35:65 containing 10 mM Na_2HPO_4 (pH adjusted to 6 with phosphoric acid), inject a 20 µL aliquot of the eluate.

HPLC VARIABLES

Column: 100 × 2 5 µm ODS Hypersil
Mobile phase: MeCN:water 40:60 containing 10 mM Na_2HPO_4, pH adjusted to 2 with orthophosphoric acid

Flow rate: 0.5
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 3.5
Internal standard: dicloxacillin

OTHER SUBSTANCES
Simultaneous: cloxacillin, floxacillin (flucloxacillin)

KEY WORDS
plasma; dicloxacillin is IS; SPE

REFERENCE
Hung,C.T.; Lim,J.K.C.; Zoest,A.R.; Lam,F.C. Optimization of high-performance liquid chromatographic analysis for isoxazolyl penicillins using factorial design, *J.Chromatogr.*, **1988**, *425*, 331–341.

SAMPLE
Matrix: blood
Sample preparation: Condition a 55 × 5 100-200 mesh AG 50W-X8 (H^+) column (Bio-Rad) with 10 mL MeCN:water 50:50. 600 µL Serum + 600 µL MeCN, vortex for 1 min, centrifuge at 2000 g for 5 min, add a 1 mL aliquot of the supernatant to the column, discard the first 200 µL effluent, collect the rest of the effluent. Remove a 450 µL aliquot and add it to 50 µL 10% sodium carbonate solution, heat at 60° for 1 h (to hydrolyse the β-lactam ring), cool in an ice bath. Remove a 100 µL aliquot and add it to 15 µL 200 mM pH 6.0 phosphate buffer, add 35 µL 80 mM 7-fluoro-4-nitrobenzo-2-oxa-1,3-diazole in MeCN, heat at 60° for 10 min, cool in an ice bath, add 30 µL 1 M HCl, inject a 5-10 µL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 ODS-80TM (Tosoh)
Mobile phase: MeOH:100 mM pH 3.0 phosphate buffer 55:45
Flow rate: 1
Injection volume: 5-10
Detector: F ex 470 em 530

CHROMATOGRAM
Retention time: 9
Limit of detection: 45 ng/mL

OTHER SUBSTANCES
Extracted: cloxacillin

KEY WORDS
derivatization; serum; SPE

REFERENCE
Iwaki,K.; Okumura,N.; Yamazaki,M.; Nimura,N.; Kinoshita,T. Precolumn derivatization technique for high-performance liquid chromatographic determination of penicillins with fluorescence detection, *J.Chromatogr.*, **1990**, *504*, 359–367.

SAMPLE
Matrix: blood
Sample preparation: 100 µL Plasma + 100 µL dicloxacillin in water + 25 µL glacial acetic acid + 2 mL ethyl acetate, vortex for 30 s, centrifuge at 2000 g for 5 min. Remove the

supernatant and evaporate it to dryness under a stream of nitrogen at 70°, reconstitute the residue in 250 μL mobile phase, inject a 10-20 μL aliquot.

HPLC VARIABLES
Column: 40 × 3.2 RP18 VeloSep (Brownlee)
Mobile phase: MeCN:10 mM pH 7 phosphate buffer 18:82
Flow rate: 1.2
Injection volume: 10-20
Detector: UV 220

CHROMATOGRAM
Retention time: 4.4
Internal standard: dicloxacillin
Limit of detection: 50 ng/mL
Limit of quantitation: 300 ng/mL

OTHER SUBSTANCES
Extracted: floxacillin
Simultaneous: phenobarbital
Noninterfering: acetaminophen, N-acetylprocainamide, amikacin, amitriptyline, caffeine, chloramphenicol, cyclosporine, digoxin, ethosuximide, gentamicin, lidocaine, nortriptyline, methotrexate, primidone, procainamide, quinidine, salicylic acid, theophylline, tobramycin, valproic acid, vancomycin, metabolites
Interfering: carbamazepine, phenytoin

KEY WORDS
plasma; dicloxacillin is IS

REFERENCE
Charles,B.G.; Foo,C.C.; Gath,J. Rapid column liquid chromatographic analysis of flucloxacillin in plasma on a microparticulate pre-column, *J.Chromatogr.B*, **1994**, *660*, 186–190.

SAMPLE
Matrix: blood, tissue, urine
Sample preparation: Plasma: Mix 50 μL MeCN and 50 μL plasma for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Urine: Mix 100 μL MeCN with 100 μL urine for 30 s, centrifuge at 5000 g for 15 min. Inject an aliquot of the supernatant. Tissue. Weigh out finely chopped tissue, suspend in 200 μL water. Mix with 200 μL MeCN, centrifuge at 10000 g for 15 min. Inject an aliquot of the supernatant.

HPLC VARIABLES
Guard column: 15 × 3.2 7 μm Newguard C18 (Alltech)
Column: 250 × 4.6 5 μm Alltima C18 (Alltech)
Mobile phase: MeCN:50 mM pH 5.0 sodium dihydrogen phosphate 30:70
Flow rate: 1.0
Detector: UV 214

CHROMATOGRAM
Retention time: 13.9
Internal standard: dicloxacillin

OTHER SUBSTANCES
Extracted: penicillin G, flucloxacillin

KEY WORDS
dicloxacillin is IS; plasma; muscle; rat

REFERENCE
Cross,S.E.; Thompson,M.J.; Roberts,M.S. Distribution of systemically administered ampicillin, benzylpenicillin, and flucloxacillin in excisional wounds in diabetic and normal rats and effects of local topical vasodilator treatment, *Antimicrob.Agents Chemother.*, **1996**, *40*, 1703–1710.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma, serum. 100 μL Plasma or serum + flucloxacillin + 100 μL 500 mM pH 2.2 citric acid buffer + 20 μL 500 mM HCl + 2.5 mL dichloromethane, extract. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 35°, reconstitute the residue in mobile phase, inject an aliquot. Urine. Dilute urine with water, inject an aliquot.

HPLC VARIABLES
Guard column: 50 × 2.1 ODS pellicular
Column: 250 × 4.6 5 μm Lichrosorb RP-8
Mobile phase: MeCN:20 mM pH 6.6 sodium acetate 34:100
Flow rate: 1
Detector: UV 220

CHROMATOGRAM
Retention time: k' 6.23
Internal standard: flucloxacillin (k' 4.19)
Limit of detection: 400 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, oxacillin, cloxacillin

KEY WORDS
plasma; serum

REFERENCE
Thijssen,H.H.W. Analysis of isoxazolyl penicillins and their metabolites in body fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *183*, 339–345.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Weigh out amount of bulk drug or capsule contents equivalent to 50 mg dicloxacillin, add 500 μL 30 mg/mL dimethyl phthalate in MeCN:water 50:50, make up to 50 mL with water, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 5 μm μBondapak C18
Mobile phase: MeOH:4% acetic acid 60:40
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 6
Internal standard: dimethyl phthalate (3.7)

OTHER SUBSTANCES
Noninterfering: excipients

KEY WORDS
capsules

REFERENCE
Hsu,M.-C.; Fann,Y.-J. Determination of cloxacillin preparations by liquid chromatography, *J.AOAC Int.*, **1992**, *75*, 26–29.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Prepare a solution of capsule contents or bulk drug in the mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher RP-18
Mobile phase: MeCN:1% acetic acid 39:61
Flow rate: 2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 4.5

OTHER SUBSTANCES
Simultaneous: degradation products, ampicillin

KEY WORDS
capsules; stability-indicating

REFERENCE
Al-Rashood,K. Simultaneous determination of ampicillin and dicloxacillin in pharmaceutical formulations by high-performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 2457–2465.

SAMPLE
Matrix: formulations
Sample preparation: Blend tablets and capsules with water in a high-speed blender for 5 min, filter, dilute with mobile phase, inject a 20 μL aliquot. Dilute oral suspensions and injections with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 70 mm long Co:Pell ODS
Column: 300 × 4.6 10 μm Chromegabond C18 (E.S. Industries)
Mobile phase: MeCN:MeOH:10 mM KH_2PO_4 19:11:70
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 26.8
Limit of detection: 3640 ng/mL

OTHER SUBSTANCES
Simultaneous: amoxicillin, ampicillin, cloxacillin, methicillin, nafcillin, oxacillin, penicillin G, penicillin V

KEY WORDS
tablets; capsules; oral suspensions; injections

REFERENCE
Briguglio,G.T.; Lau-Cam,C.A. Separation and identification of nine penicillins by reverse phase liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1984**, *67*, 228–231.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out contents of amoxicillin/dicloxacillin capsules equivalent to 100 mg amoxicillin, add 10 mL water, stir magnetically for 10 min, filter, discard first 5 mL of the filtrate. 5 mL filtrate + 10 mL 1 mg/mL albuterol sulfate in water, make up to 100 mL with water, filter (0.45 μm), inject a 10 μL aliquot of the filtrate.

HPLC VARIABLES
Column: 200 × 4.6 10 μm LiChrosorb RP-8
Mobile phase: MeOH:20 mM ammonium acetate 50:50, pH adjusted to 5 with acetic acid
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 6.066
Internal standard: albuterol sulfate (3.388)

OTHER SUBSTANCES
Simultaneous: amoxicillin

KEY WORDS
capsules

REFERENCE
el Walily,A.F.M.; el-Anwar,F.; Eid,M.A.; Awaad,H. High-performance liquid chromatographic and derivative ultraviolet spectrophotometric determination of amoxycillin and dicloxacillin mixtures in capsules, *Analyst*, **1992**, *117*, 981–984.

SAMPLE
Matrix: milk
Sample preparation: Mix 10 mL milk with 2 mL 100 mM tetraethylammonium chloride, add 40 mL MeCN slowly with continual stirring, let stand for 10 min, decant the supernatant through a plug of glass wool. Collect 40 mL filtrate, add 2 mL buffer, evaporate to 1-2 mL under reduced pressure at 40-50°, dilute to 4 mL with water, filter (0.45 μm PVDF). Inject a 2 mL aliquot onto a 150 × 4.6 5 μm Supelcosil LC-18 column, elute with MeCN:10 mM KH_2PO_4 0:100 for 3 min, to 60:40 over 37 min at 1 mL/min, collect a 1.5-2 mL aliquot containing the compound (ca. 30.5 min), evaporate to <1 mL under reduced pressure, make up to 1 mL with water, inject an aliquot. (Prepare the buffer by mixing 10 mM KH_2PO_4 and 10 mM Na_2HPO_4 in a 5:1 ratio, pH 6.)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Supelcosil LC-18-DB
Mobile phase: MeCN:buffer 38:62 (Buffer was 2 mM phosphoric acid containing 8 mM potassium dihydrogen phosphate.)
Flow rate: 1
Injection volume: 200
Detector: UV 215

REFERENCE
Moats,W.A.; Romanowski,R.D. Multiresidue determination of β-lactam antibiotics in milk and tissues with the aid of high-performance liquid chromatographic fractionation for clean up, *J.Chromatogr.A*, **1998**, *812*, 237–247.

SAMPLE
Matrix: milk

Sample preparation: Condition a 3 mL 500 mg Baker-10 C18 SPE cartridge (J.T. Baker) with 3 mL MeOH and 3 mL distilled water. Add 20 mL MeCN to 10 mL milk, vortex for 1 min, centrifuge at 1500 g for 10 min, concentrate the supernatant to 2-3 mL on a rotary evaporator at 40°, add to the SPE cartridge, dry the cartridge under reduced pressure for 3 min, elute with 1 mL MeOH, filter (0.45 μm) the eluate, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Kaseisorb LC ODS-300-5 (Tokyo Kasei)
Mobile phase: MeCN:MeOH:50 mM KH_2PO_4 buffer 20:10:80 containing 5 mM sodium 1-decanesulfonate, adjusted to pH 3.5 with concentrated phosphoric acid
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM

Retention time: 30
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: ampicillin, cloxacillin, nafcillin, penicillin G

KEY WORDS

SPE

REFERENCE

Takeba,K.; Fujinuma,K.; Miyazaki,T.; Nakazawa,H. Simultaneous determination of β-lactam antibiotics in milk by ion-pair liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 205–211.

SAMPLE

Matrix: milk
Sample preparation: Condition a 6 mL 500 mg Bond Elut C18 SPE cartridge with 10 mL MeOH, 10 mL water, 5 mL 2% NaCl, and 5 mL 100 mM pH 8 phosphate extraction buffer. Add 30 mL 100 mM pH 8 phosphate extraction buffer to 5 mL milk, add 1.65 mL 1 M sulfuric acid to reach pH 4.0-4.5, vortex for 30 s, centrifuge at 2400 g for 10 min, add 600 μL 5 M NaOH to the supernatant to reach pH 8, vortex, centrifuge at 2400 g for 5 min. Add the supernatant to a reservoir attached to the SPE cartridge, pull through the SPE cartridge at 3 mL/min, remove the reservoir and elute with 1 mL MeCN:water 40:60. Add 500 μL derivatizing reagent to the eluate, vortex, heat at 65° for 10 min, cool to room temperature (protect from light), inject a 100 μL aliquot of the derivatized sample. (Prepare the 100 mM pH 8 phosphate extraction buffer as follows. Dissolve 15.6 g K_2HPO_4 dihydrate in 800 mL water, adjust pH to 8 with 10 M NaOH, make up to 1 L. Prepare the derivatizing reagent as follows. Weigh out 13.78 g 1,2,4-triazole, add 60 mL water, stir, add 10 mL 100 mM mercuric chloride solution, mix, adjust pH to 9.0 ± 0.5 with 5 M NaOH, dilute to 100 mL with water.)

HPLC VARIABLES

Column: 150 × 3.9 5 μm Symmetry C8 (Waters)
Mobile phase: MeCN:MeOH:buffer 37:5:58 (Prepare the 100 mM pH 6.5 phosphate buffer containing 15 mM thiosulfate and 30 mM tetrabutylammonium hydrogen sulfate as follows. Weigh 4.969 g anhydrous NaH_2PO_4, 10.139 g Na_2HPO_4 dihydrate, 3.894 g sodium thiosulfate pentahydrate, and 10.186 g tetrabutylammonium hydrogen sulfate, dissolve in 800 mL water, adjust pH to 6.5 with 5 M NaOH, dilute to 1 L with water, mix thoroughly, filter under vacuum (0.45 μm).)
Flow rate: 1
Injection volume: 100
Detector: UV 340

CHROMATOGRAM
Retention time: 17
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: oxacillin, cloxacillin

KEY WORDS
derivatization; SPE

REFERENCE
Verdon,E.; Couedor,P. Determination of isoxazolylpenicillins residues in milk by ion-pair reversed-phase high-performance liquid chromatography after precolumn derivatization, *J.Chromatogr.B*, **1998**, *705*, 71–78.

SAMPLE
Matrix: milk
Sample preparation: 50 g Milk + 2 drops penicillinase (Difco Laboratories), let stand 1 h at 37°, add 50 MeCN, shake vigorously for 1 min, centrifuge at 9000 g for 10 min, decant, add 5 g NaCl, swirl to dissolve, add 100 mL dichloromethane, shake for 1 min, centrifuge at 1000 g for 10 min. Remove top aqueous layer and extract organic layer with 25 mL 10% NaCl by shaking and centrifuging as before. Combine aqueous layers, add 1 mL 0.3% mercuric chloride in water, let stand 30 min, add 1 mL 2 M HCl, extract with three 50 mL portions of dichloromethane by shaking each portion for 1 min and centrifuging at 1000 g for 10 min, filter dichloromethane extracts through 30 g anhydrous sodium sulfate, evaporate to dryness under reduced pressure at 35°, if water remains add 5-10 mL MeOH to flask and complete evaporation. Dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 2 mL IS solution, inject a 20 μL aliquot. (Prepare IS solution by dissolving 10 μL benzaldehyde in 100 mL dichloromethane, evaporate 1 mL to dryness under reduced pressure, dissolve residue in 1 mL 10% acetic acid, add 0.5 mL 0.08% dansyl hydrazine in 10% acetic acid, let stand 90 min to overnight in the dark, transfer reaction mixture to a separatory funnel with three 25 mL portions of dichloromethane, add 5 mL 2 M HCl, shake for 1 min, wash organic layer with 5 mL 5% $NaHCO_3$ solution, filter through 10-20 g anhydrous sodium sulfate. Extract acid aqueous layer again with 25 mL dichloromethane. Combine dichloromethane layers and evaporate to dryness at 35° under reduced pressure. Dissolve residue in 100 mL MeCN then dilute an aliquot 1:4 with MeCN.)

HPLC VARIABLES
Column: 250 × 4 10 μm Lichrosorb RP-18
Mobile phase: MeCN:water 58:42
Flow rate: 1
Injection volume: 20
Detector: F ex 254 em 500 filter

CHROMATOGRAM
Retention time: 10.73
Internal standard: benzaldehyde (derivatized) (12.18)
Limit of detection: 5 ng/g

OTHER SUBSTANCES
Simultaneous: penicillin G, methicillin, oxacillin, cloxacillin, penicillin V, nafcillin, phenethicillin

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Shimoda,W.; Roybal,J.E.; Vieira,C. Multiresidue method for determination of eight neutral β-lactam penicillins in milk by fluorescence-liquid chromatography, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 968–971.

SAMPLE
Matrix: milk
Sample preparation: Add 2 volumes MeCN to milk, stand 5 min, decant aqueous portion, suction filter, extract with an equal volume of 1:1 methylene chloride:hexane, centrifuge aqueous phase at 3000 rpm for 10 min. Dilute 3:1 with 20 mM sodium acetate buffer and filter (0.2 μm nylon). Inject 50 μL onto column with mobile phase A, run mobile phase A for 30 min and elute to waste. After 30 min switch to mobile phase B and elute through detector.

HPLC VARIABLES
Column: 100 × 8 Radial-Pak 10 μm μBondapak C18
Mobile phase: A 20 mM sodium acetate buffer; B Gradient. MeCN:MeOH:20 mM sodium acetate buffer from 15:10:75 to 30:0:70 over 15 min and hold at 30:0:70
Flow rate: A 3; B 2
Injection volume: 50
Detector: E, Waters 464 pulsed electrochemical detector using a thin layer cell with a Ag/AgCl reference electrode. E1 = 1300 mV for 0.166 s, E2 = 1500 mV for 0.166 s, E3 = -200 mV for 0.333 s.

CHROMATOGRAM
Retention time: 21
Limit of detection: 0.3 ppm

OTHER SUBSTANCES
Simultaneous: penicillin V, ampicillin, methicillin, penicillin G, oxacillin, nafcillin, cloxacillin.

REFERENCE
Kirchmann,E.; Earley,R.L.; Welch,L.E. The electrochemical detection of penicillins in milk, *J.Liq.Chromatogr.*, **1994**, *17*, 1755–1772.

SAMPLE
Matrix: milk
Sample preparation: Condition a Bond Elut C8 SPE cartridge with 5 mL MeOH and 5 mL water. 20 mL Milk + 20 mL buffer, heat at 60° for 20 min or until milk curdles, centrifuge for 10 min, add the supernatant to the SPE cartridge, wash with two 2.5 mL portions of water, elute with 2.5 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, extract the residue with three 100 μL portions of 50 mM pH 6.0 potassium phosphate buffer, filter (0.2 μm), inject an aliquot of the filtrate. (Buffer was 545 mL 100 mM citric acid, 455 mL 200 mM Na_2HPO_4, and 74.4 g EDTA, adjust to pH 4.5 with ammonium hydroxide, make up to 2 L with water.)

HPLC VARIABLES
Column: 250 × 4.6 10 μm Lichrosorb RP-8
Mobile phase: MeOH:50 mM pH 6.0 potassium phosphate buffer 35:65
Flow rate: 1
Injection volume: 200
Detector: UV 210 or Charm II assay

CHROMATOGRAM
Retention time: 74.90

OTHER SUBSTANCES
Extracted: ampicillin, ceftiofur, cephapirin, cloxacillin, nafcillin, oxacillin, penicillin G
Simultaneous: amoxicillin

KEY WORDS
SPE

REFERENCE
Zomer,E.; Quintana,J.; Saul,S.; Charm,S.E. LC-Receptograms: A method for identification and quantitation of β-lactams in milk by liquid chromatography with microbial receptor assay, *J.AOAC Int.*, **1995**, *78*, 1165–1172.

SAMPLE
Matrix: milk
Sample preparation: Condition a 500 mg tC18 SPE cartridge (Waters) with 20 mL MeOH, 20 mL water, and 10 mL 2% NaCl. Centrifuge 30 mL milk at 1500 g for 10 min. Dilute a 10 mL portion of the defatted milk with 20 mL water, add 200 μL 2 μg/mL penicillin V in pH 9.0 buffer, add 6 mL 170 mM sulfuric acid, add 5.6 mL 5% sodium tungstate, shake vigorously for 1 min, allow to stand for 5 min, check that the pH is in the range 4.6-4.8 (if it is outside this range start again using a different volume of sodium tungstate solution), centrifuge at 1500 g for 10 min, adjust the pH of the supernatant to 8.1-8.2 with 5 M and 0.1 M NaOH, filter (glass fiber) the clear liquid phase. Pass the filtrate through the SPE cartridge at 2 mL/min, wash with 2 mL water, dry by pulling air through the cartridge for 1 min, elute with 2 mL MeCN. Add 150 μL pH 9.0 buffer to the eluate and evaporate to about 100 μL under a stream of nitrogen at 45-50°, add 400 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, use 500 μL water to transfer the mixture to a separatory funnel, add 20 mL dichloromethane, add 5 mL pH 2.45 buffer, shake for 1 min, let stand for no more than 5 min. Remove the organic layer and evaporate it to dryness under reduced pressure at 35-40°, dissolve the residue in 500 μL pH 9.0 buffer, add 75 μL reagent I, vortex for 30 s, let stand at room temperature for 10 min, add 450 μL reagent II, vortex for 1 min, heat at 55 ± 1° for 30 min, cool, filter (0.45 μm), inject a 150 μL aliquot. (Prepare pH 9.0 buffer by dissolving 0.34 g KH_2PO_4 in water, adjusting the pH to 9.0 with NaOH, and making up to 100 mL with water. Prepare pH 2.45 buffer by dissolving 2.72 g KH_2PO_4 in water, adjusting the pH to 2.45 with phosphoric acid, and making up to 100 mL with water. Prepare reagent 1 by dissolving 1.13 g benzoic anhydride in MeCN, make up to 25 mL with MeCN. Prepare reagent II by dissolving 6.905 g 1,2,4-triazole in 30 mL water and adding 5 mL 26 mM mercuric chloride in water, adjust pH to 9.0 ± 0.05 with 5 M NaOH, make up to 50 mL. Prepare reagents I and II 1-4 h before use. Silanize glassware with dichlorodimethylsilane.)

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak C18
Mobile phase: Gradient. A as MeCN:buffer 10:90. B was MeCN:buffer 30:70. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 13 min, return to initial conditions over 2 min, re-equilibrate at initial conditions for 5 min. (Prepare buffer by dissolving 9.938 g Na_2HPO_4, 17.938 g $NaH_2PO_4.H_2O$, and 4.964 g sodium thiosulfate in water, make up to 2 L with water, pH 6.5.)
Column temperature: 30
Flow rate: 1
Injection volume: 150
Detector: UV 323

CHROMATOGRAM
Retention time: 40

Internal standard: penicillin V (28.5)
Limit of detection: 2.7 ng/mL
Limit of quantitation: 3.7 ng/mL

OTHER SUBSTANCES
Extracted: amoxicillin, ampicillin, cloxacillin, oxacillin, penicillin G

KEY WORDS
derivatization; cow; SPE

REFERENCE
Sorensen,L.K.; Rasmussen,B.M.; Boison,J.O.; Keng,L. Simultaneous determination of six penicillins in cows' raw milk by a multiresidue high-performance liquid chromatographic method, *J.Chromatogr.B*, **1997**, *694*, 383–391.

SAMPLE
Matrix: solutions
Sample preparation: Prepare an aqueous solution, inject a 200 μL aliquot.

HPLC VARIABLES
Guard column: present but not specified
Column: 150 × 4.6 4 μm Micropak SPC-18 C18
Mobile phase: Gradient. MeCN:10 mM orthophosphoric acid from 15:85 to 60:40 over 20 min
Flow rate: 1
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Retention time: 20

OTHER SUBSTANCES
Simultaneous: methicillin, penicillin G, penicillin V, cloxacillin, nafcillin, carbenicillin

REFERENCE
Moats,W.A. Effect of the silica support of bonded reversed-phase columns on chromatography of some antibiotic compounds, *J.Chromatogr.*, **1986**, *366*, 69–78.

SAMPLE
Matrix: solutions
Sample preparation: React the antibiotic, triethylamine, and 1-(2,5-dihydroxyphenyl)-2-bromoethanone in a 1:2:4 molar ratio in DMF at 45° for 2 h (use dibenzo-18-crown-6 to make the sodium salt soluble), inject a 10 μL aliquot. (Preparation of 1-(2,5-dihydroxyphenyl)-2-bromoethanone is as follows. Stir 27.6 g 1,4-dimethoxybenzene and 28 mL bromoacetyl bromide at 0°, add 53.4 g aluminum bromide over 10 min (an exothermic reactions ensues), let stand at room temperature for 12 h, add 100 mL 48% HBr, add 100 g ice, stir for 1 h, extract twice with 200 mL portions of diethyl ether. Combine the extracts and wash them 3 times with 200 mL portions of water, dry over 40 g anhydrous magnesium sulfate, evaporate to dryness, recrystallize the product 3 times from EtOH to yield 1-(2,5-dihydroxyphenyl)-2- bromoethanone monobromoacetate (mp 105-107°). Dissolve 11 g 1-(2,5-dihydroxyphenyl)-2-bromoethanone monobromoacetate in 200 mL warm dry MeOH saturated with HBr, stir for 18 h, add 200 mL water, cool to -10°. Collect the yellow solid and dry it under vacuum at 50° for 48 h, recrystallize from toluene:heptane 50:50 then toluene to obtain 1-(2,5-dihydroxyphenyl)-2-bromoethanone as yellow needles (mp 117-119°).)

HPLC VARIABLES
Column: 250 × 4 7 μm RP-18 LiChrocart (Merck)

Mobile phase: MeOH:100 mM pH 6.5 sodium acetate 58:42
Flow rate: 1
Injection volume: 10
Detector: E, Bioanalytical Systems Model LC4B, glassy carbon electrode 0.8 V, Ag/AgCl reference electrode

CHROMATOGRAM
Retention time: 28.8

OTHER SUBSTANCES
Simultaneous: carbenicillin, cephapirin, cloxacillin, hetacillin, methicillin, nafcillin, oxacillin, penicillin G

KEY WORDS
derivatization

REFERENCE
Munns,R.K.; Roybal,J.E.; Shimoda,W.; Hurlbut,J.A. 1-(4-Hydroxyphenyl)-, 1-(2,4-dihydroxyphenyl)- and 1-(2,5-dihydroxyphenyl)-2-bromoethanones: new labels for determination of carboxylic acids by high-performance liquid chromatography with electrochemical and ultraviolet detection, *J.Chromatogr.*, **1988**, *442*, 209–218.

SAMPLE
Matrix: solutions
Sample preparation: Separate buffer containing drug from human serum albumin by centrifuging at 37° at 700 g for 3 min using a Micropartition System MPS-1 (Amicon) unit, inject a 10-20 μL aliquot of the ultrafiltrate.

HPLC VARIABLES
Guard column: C18/Corasil (Waters)
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:10 mM ammonium acetate 25:65
Flow rate: 1.5
Injection volume: 10-20
Detector: UV 220

REFERENCE
Terasaki,T.; Nouda,H.; Tsuji,A. Relationship between lipophilicity and binding affinity with human serum albumin for penicillin and cephem antibiotics, *J.Pharmacobiodyn.*, **1992**, *15*, 99–106.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Ultra-Turrax) 25 g tissue with 25 mL MeCN for 1 min, add 5 mL 500 mM pH 2.2 phosphate buffer while the homogenizer is still running, add 65 mL MeCN, homogenize for 1 min, centrifuge at 4000 g for 10 min. Remove the supernatant and add it to 7 g NaCl and 50 mL dichloromethane, shake for 2 min, allow to stand for 30 min. Remove the upper organic layer and add it to 5 g anhydrous sodium sulfate, shake for 30 s, filter through a cotton-wool plug, evaporate to about 4 mL under reduced pressure at 30°, add 3 mL dichloromethane, evaporate to about 4 mL, add 3 mL light petroleum, evaporate to about 0.5 mL, Suspend this residue with sonication in three 3 mL portions of light petroleum and place these fractions in a separate tube, rinse the original tube with 2 mL pH 7 phosphate buffer. Add the phosphate buffer rinse to the light petroleum extracts, vortex for 30 s, centrifuge, remove the aqueous layer. Extract the light petroleum layer with 2 mL pH 7 phosphate buffer and with two 1.5 mL portions of pH 7 phosphate buffer, combine all the aqueous phase, centrifuge, inject a 200 μL aliquot on to column A and elute to waste with mobile phase B, after 15 min elute to waste with mobile phase C at 2 mL/min, after 10 min elute the contents of column A on

to column B with mobile phase D, after 2 min remove column A from the circuit, elute column B with mobile phase D, monitor the effluent from column B. (Wash column A with mobile phase A at 2 mL/min for 7 min, with mobile phase A at 1 mL/min for 5 min, with mobile phase B at 2 mL/min for 8 min, and with mobile phase B at 1 mL/min for 6 min.)

HPLC VARIABLES

Column: A 4 × 4 5 μm LiChrospher 100 RP-18e; B 250 × 4 5 μm LiChrospher 100 RP-18e
Mobile phase: A MeCN:water 50:50; B 20 mM pH 7 phosphate buffer; C MeCN:20 mM pH 3 phosphate buffer 10:90; D MeCN:200 mM pH 3.0 phosphate buffer 35:65 containing 2 mM disodium EDTA
Column temperature: 35
Flow rate: 1 (except where indicated)
Injection volume: 200
Detector: E, Merck Model L3500, glassy carbon working electrode +0.65 V, stainless-steel auxiliary electrode, Ag/AgCl reference electrode following post-column reaction. The column effluent flowed through a 10 m × 0.3 mm ID woven PTFE coil illuminated by a UV 254 low-pressure mercury lamp to the detector.

CHROMATOGRAM

Retention time: 13.0
Limit of detection: 4.6 ng

OTHER SUBSTANCES

Extracted: cloxacillin, oxacillin, penicillin V, penicillin G

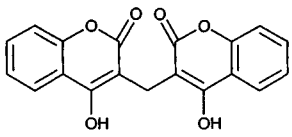
KEY WORDS

post-column reaction; post-column photochemical derivatization; cow; muscle; column-switching

REFERENCE

Lihl,S.; Rehorek,A.; Petz,M. High-performance liquid chromatographic determination of penicillins by means of automated solid-phase extraction and photochemical degradation with electrochemical detection, *J.Chromatogr.A*, **1996**, *729*, 229–235.

Dicumarol



Molecular formula: $C_{19}H_{12}O_6$
Molecular weight: 336.30
CAS Registry No.: 66-76-2
Merck Index: 3140
Lednicer No.: 1 147

SAMPLE

Matrix: formulations
Sample preparation: Powder tablets, make a 250 μg/mL solution in 10 mM NaOH. Remove a 5 mL aliquot and make it up to 25 mL with mobile phase, filter, inject an aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: THF:MeOH:water:acetic acid 35:10:65:0.1
Flow rate: 1.5
Injection volume: 20
Detector: UV 311

OTHER SUBSTANCES

Also analyzed: warfarin, phenprocoumon

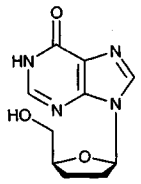
KEY WORDS

tablets

REFERENCE

Moore,E.S. Liquid chromatographic determination of coumarin anticoagulants in tablets: collaborative study, *J.Assoc.Off.Anal.Chem.*, **1987**, *70*, 834–836.

Didanosine



Molecular formula: $C_{10}H_{12}N_4O_3$
Molecular weight: 236.23
CAS Registry No.: 69655-05-6
Merck Index: 3148
Lednicer No.: 5 146

SAMPLE

Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and 2 mL water. 50 μL Plasma or amniotic fluid + 50 μL 5 μg/mL 3-hydroxyacetamidophenol in water, add to SPE cartridge, wash twice with 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate under vacuum, reconstitute the residue in 100 μL MeCN:water 6:94, inject a 50 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Ultrasphere ODS C18
Mobile phase: Gradient. A was MeCN:pH 4.0 ammonium phosphate buffer 6:94. B was MeCN:pH 4.0 ammonium phosphate buffer 25:75. A:B from 100:0 to 100:0 over 20 min, to 100:0 over 5 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM

Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES

Extracted: antipyrine

KEY WORDS

plasma; monkey; pharmacokinetics; SPE

REFERENCE

Pereira,C.M.; Nosbisch,C.; Winter,H.R.; Baughman,W.L.; Unadkat,J.D. Transplacental pharmacokinetics of dideoxyinosine in pigtailed macaques, *Antimicrob.Agents Chemother.*, **1994**, *38*, 781–786.

SAMPLE

Matrix: blood
Sample preparation: Condition a 3 mL Supelclean C18 SPE cartridge with MeOH, water, and two aliquots of 10 mM pH 8.0 phosphate buffer. Add 500 μL plasma then 1 mL 5 μg/mL IS in Na_2HPO_4 buffer to the SPE cartridge. Wash with 10 mM pH 8.0 phosphate buffer and water. Dry cartridge under vacuum and elute with 2 aliquots of MeOH. Evaporate the eluate to dryness and reconstitute the residue in 10 mM pH 8.0 phosphate buffer. Inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 20 × 4.6 5 μm Supelguard LC-18s
Column: 250 × 4.6 5 μm Supelcosil LC-18s
Mobile phase: MeOH:100 mM pH 5.0 phosphate buffer 20:80
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Internal standard: d4C

KEY WORDS
dog; plasma; pharmacokinetics; SPE

REFERENCE
Sinko,P.J.; Sutyak,J.P.; Leesman,G.D.; Hu,P.; Makhey,V.D.; Yu,H.; Smith,C.L. Oral absorption of anti-AIDS nucleoside analogues: 3. Regional absorption and in vivo permeability of 2',3'-dideoxyinosine in an intestinal-vascular access port (IVAP) dog model, *Biopharm.Drug Dispos.*, **1997**, *18*, 697–710.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bakerbond C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. Add 500 μL plasma to the SPE cartridge, wash with 1 mL water, allow to dry under vacuum for 10 min, elute with two 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex for 30 s, centrifuge at 800 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 LiChroCART 4-4 RP-8 (Merck)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeOH:5 mM pH 6.8 phosphate buffer 10:90
Flow rate: 1
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 10.5
Internal standard: didanosine

OTHER SUBSTANCES
Extracted: stavudine

KEY WORDS
plasma; rat; human; didanosine is IS; SPE

REFERENCE
Burger,D.M.; Rosing,H.; van Gijn,R.; Meenhorst,P.L.; van Tellingen,O.; Beijnen,J.H. Determination of stavudine, a new antiretroviral agent, in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1992**, *584*, 239–247.

SAMPLE
Matrix: blood
Sample preparation: Condition a Toyopak ODS M SPE cartridge (Tosoh, Tokyo) with 6 mL MeOH, 12 mL water, and 2 mL 100 mM pH 4.5 phosphate buffer. 100 μL Plasma + 10 μL 85 μM IS + 890 μL 100 mM pH 4.5 phosphate buffer, mix, add to the SPE cartridge at 120-150 μL/min, wash with 2 mL 100 mM ph 4.5 phosphate buffer, wash with 2 mL water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 100 μL dry pyridine, add 900 μL 3 mM reagent in dry benzene (Caution! Benzene is a carcinogen!), heat at 100° in the dark for 50 min, cool, evaporate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL mobile phase, inject a 20 μL aliquot. (The reagent was 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (Dojindo Laboratories, Kumamoto, Japan). Synthesis is as follows. Add ethyl oxalyl chloride in ether to a solution of diazomethane in ether at 0° to give ethyl diazopyruvate (Caution! Diazo compounds are explosive and toxic!) (cf. Bueh-

ler,C.A.; Pearson,D.E. Survey of Organic Syntheses, Wiley, New York, 1970, p. 179). Heat 100 mg ethyl diazopyruvate, a few mg copper(II) acetylacetonate, and 400 μL chloroacetonitrile in benzene at 60° overnight (Caution! Benzene is a carcinogen!), cool, add to sodium bicarbonate solution, extract with ether, dry the organic layer, evaporate, chromatograph on silica with petroleum ether:ethyl acetate 90:10, distil the product at 90°/12 mm Hg to give ethyl 2-chloromethyl-5-oxazolecarboxylate as an oil in 18% yield (US Patent 4 603 209 (July 29, 1986)). Add 2 mL phosphorus oxychloride dropwise to a solution of 2 g sesamol in 3 mL DMF at 0°, heat on a steam bath with frequent shaking for 1 h, cool in ice, add 50 mL saturated sodium acetate solution, heat on a steam bath for 30 min, cool, filter, recrystallize the solid from EtOH to give 2-hydroxy-4,5-methylenedioxybenzaldehyde as colorless needles (mp 125-126°) (Bull. Chem. Soc. Jpn. 1962, 35, 1321). Stir 1.4 g ethyl 2-chloromethyl-5-oxazolecarboxylate, 1.5 g 2-hydroxy-4,5-methylenedioxybenzaldehyde, 2 g potassium carbonate, and 50 mL anhydrous DMF at 120° overnight, cool, filter. Evaporate the filtrate to dryness under reduced pressure to give 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 186°) (yield 39%). Reflux 260 mg 2-(5-ethoxycarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran, 100 mg KOH, 20 mL EtOH, and 30 mL water for 2 h, concentrate under reduced pressure, dissolve the residue in 100 mL water, wash with ethyl acetate, treat the aqueous layer with activated carbon, acidify the aqueous layer to pH 2 with 2 M HCl. Filter the precipitate and recrystallize it from EtOH to give 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran as a colorless crystalline powder (mp 294-295°). Reflux 150 mg 2-(2-oxazole-5-carboxylic acid)-5,6-methylenedioxybenzofuran and 5 mL thionyl chloride for 2 h, pour the reaction mixture into 300 mL petroleum ether. Filter the precipitate and dry it over KOH to give 2-(5-chlorocarbonyl-2-oxazolyl)-5,6-methylenedioxybenzofuran (mp 290°) (Anal. Sci. 1989, 5, 525).)

HPLC VARIABLES

Column: 150 × 4.6 5 μm TSKgel ODS-80TM
Mobile phase: MeCN:100 mM pH 7.0 phosphate buffer 35:65
Flow rate: 1
Injection volume: 20
Detector: F ex 360 em 475

CHROMATOGRAM

Retention time: 14.6
Internal standard: 3'-deoxythymidine (21.5)
Limit of detection: 1.3 pmole

OTHER SUBSTANCES

Extracted: 2',3'-dideoxyadenosine

KEY WORDS

derivatization; rat; plasma; pharmacokinetics; SPE

REFERENCE

Nagaoka,H.; Nohta,H.; Saito,M.; Ohkura,Y. Determination of 2',3'-dideoxyinosine and 2',3'-dideoxyadenosine in rat plasma by high-performance liquid chromatography with precolumn fluorescence derivatization, *Chem.Pharm.Bull.(Tokyo)*, **1992**, *40*, 2202–2204.

SAMPLE

Matrix: blood
Sample preparation: 200 μL Plasma + 400 μL ethyl acetate:MeCN 50:50, vortex for 30 s. Remove a 200 μL aliquot of the supernatant and evaporate it to dryness under a stream of nitrogen at 50°, reconstitute the residue in 200 μL mobile phase, vortex for 10 s, inject a 10 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 5 μm Lichrospher 60 RP-select B

Column: 125 × 4 5 μm Lichrospher 60 RP-select B
Mobile phase: MeOH:pH 7.0 phosphate buffer 5:95
Flow rate: 1
Injection volume: 10
Detector: UV 250

OTHER SUBSTANCES
Also analyzed: zalcitabine (UV 270)

KEY WORDS
plasma; rabbit; pharmacokinetics

REFERENCE
Mirchandani,H.L.; Chien,Y.W. Intestinal absorption of dideoxynucleosides: Characterization using a multiloop in situ technique, *J.Pharm.Sci.*, **1995**, *84*, 44–48.

SAMPLE
Matrix: blood
Sample preparation: Filter (Millipore Ultrafree-MC, 10000 molecular mass limit) 250 μL serum while centrifuging at 17000 g for 1.5 h, inject a 50 μL aliquot of the clear ultrafiltrate.

HPLC VARIABLES
Column: 150 × 3.9 4 μm Nova-Pak phenyl
Mobile phase: Isopropanol:20 mM pH 5 sodium citrate 2.5:97.5
Flow rate: 1
Injection volume: 50
Detector: UV 250

CHROMATOGRAM
Retention time: 5.32
Limit of detection: 25 ng/mL

OTHER SUBSTANCES
Extracted: zalcitabine, zidovudine

KEY WORDS
serum; ultrafiltrate

REFERENCE
Rosell-Rovira,M.L.; Pou-Clavé,L.; Lopez-Galera,R.; Pascual-Mostaza,C. Determination of free serum didanosine by ultrafiltration and high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *675*, 89–92.

SAMPLE
Matrix: blood, CSF
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 6 mL MeOH and 12 mL water. 1 mL Plasma or CSF + 2'-deoxyadenosine, add to the SPE cartridge, wash with 1 (CSF) or 2 (plasma) mL water, elute slowly with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 200 μL water, filter (Ultrafree-MC 0.45 μm) while centrifuging, inject an aliquot of the filtrate. (CSF can also be injected directly.)

HPLC VARIABLES
Column: 100 × 8 Nova-Pak radial compression
Mobile phase: MeOH:10 mM ammonium phosphate 10:90
Flow rate: 1.5

Detector: UV 248

CHROMATOGRAM
Retention time: 4.6
Internal standard: 2'-deoxyadenosine (5.3)
Limit of detection: 100 nM

OTHER SUBSTANCES
Extracted: 2',3'-dideoxyadenosine (UV 260)

KEY WORDS
plasma; monkey; SPE; pharmacokinetics

REFERENCE
Hawkins,M.E.; Mitsuya,H.; McCully,C.M.; Godwin,K.S.; Murakami,K.; Poplack,D.G.; Balis,F.M. Pharmacokinetics of dideoxypurine nucleoside analogs in plasma and cerebrospinal fluid of Rhesus monkeys, *Antimicrob.Agents Chemother.*, **1995**, *39*, 1259–1264.

SAMPLE
Matrix: blood, cell suspensions, perfusate
Sample preparation: Centrifuge cellular suspensions at 17000 g for 5 min, inject a 25 μL aliquot. Centrifuge perfusion fluid at 17000 g for 5 min, inject a 50 μL aliquot. Dilute 1 mL plasma with 1 mL saturated ammonium sulfate, vortex for 30 s, centrifuge at 3000 g for 2 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 250 × 4 5 μm Phenyl Hypersil NC-04
Mobile phase: MeOH:1.4 g/L sodium acetate 15:85, adjusted to pH 6.55
Flow rate: 1
Injection volume: 25-50
Detector: UV 250

CHROMATOGRAM
Retention time: 8
Limit of detection: 25 ng/mL

KEY WORDS
plasma

REFERENCE
Frijus-Plessen,N.; Michaelis,H.C.; Foth,H.; Kahl,G.F. Determination of 3'-azido-3'-deoxythymidine, 2',3'-dideoxycytidine, 3'-fluoro-3'-deoxythymidine and 2',3'-dideoxyinosine in biological samples by high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *534*, 101–107.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a C18 SPE cartridge with two 1 mL portions of MeOH and two 1 mL portions of 100 mM pH 6.9 phosphate buffer. 100 μL Plasma + 400 μL 10 μg/mL IS in 100 mM pH 6.9 phosphate buffer, add to the SPE cartridge, let stand for 1 min, wash with two 1 mL portions of 100 mM pH 6.9 phosphate buffer, elute with 1 mL MeOH:5 mM pH 6.9 phosphate buffer 75:25, concentrate the eluate to about 150 μL under a stream of nitrogen, inject an aliquot. Urine. 100 μL Urine + 400 μL 10 μg/mL IS in 100 mM pH 6.9 phosphate buffer, mix, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18

Mobile phase: MeCN:10 mM phosphate buffer adjusted to pH 6.9 with dilute phosphoric acid 4:96
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 280

CHROMATOGRAM
Retention time: 8
Internal standard: 5'-deoxy-5-fluorouridine (5)
Limit of detection: 200 ng/mL

OTHER SUBSTANCES
Noninterfering: metabolites

KEY WORDS
plasma; rat; pharmacokinetics; SPE

REFERENCE
Wientjes,M.G.; Au,J.L.-S. High-performance liquid chromatographic analysis of 2',3'-dideoxyinosine in biological samples, *J.Chromatogr.*, **1991**, *563*, 400–406.

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Condition a 3 mL Bond Elut SPE cartridge with one volume MeOH and one volume water. 25-400 μL Plasma + 50 μL 5 μg/mL 3-acetamidophenol in water, make up to 500 μL with water, add to SPE cartridge, wash with two 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 30-40°, reconstitute the residue in 100 μL MeCN:water 6:94, inject a 50 μL aliquot. Urine. 1 mL Urine + 49 mL water + 1 mL 100 μg/mL 3-acetamidophenol, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 30 × 2.1 C18 (Alltech)
Column: 250 × 4.6 Ultrasphere C18
Mobile phase: MeCN:50 mM pH 4.0 ammonium phosphate 6:94
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 7.2
Internal standard: 3-acetamidophenol (14.0)
Limit of detection: 500 ng/mL (plasma)

KEY WORDS
plasma; monkey; SPE; pharmacokinetics

REFERENCE
Ravasco,R.J.; Unadkat,J.D.; Tsai,C.C. A high-performance liquid chromatographic assay for dideoxyinosine in monkey plasma and urine, *J.Pharm.Sci.*, **1992**, *81*, 690–691.

SAMPLE
Matrix: cell suspensions
Sample preparation: 1 mL Cell suspension + 500 μL ice-cold MeCN + 500 μL water, centrifuge. Remove the supernatant and evaporate it to dryness, reconstitute the residue in 200 μL water, inject an aliquot.

HPLC VARIABLES

Column: Partisil-10 SAX
Mobile phase: Gradient. A was 30 mM pH 4.8 ammonium phosphate. B was MeCN:700 mM pH 4.6 ammonium phosphate 10:90. A:B 100:0 for 5 min then a convex gradient to 75:25 over 10 min then a convex gradient to 0:100 over 15 min, stay at 0:100 for 15 min
Flow rate: 1.7
Detector: UV 254

CHROMATOGRAM

Retention time: 9.0

OTHER SUBSTANCES

Extracted: metabolites, ATP

REFERENCE

Mukherji,E.; Au,J.L.-S.; Mathes,L.E. Differential antiviral activities and intracellular metabolism of 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine in human cells, *Antimicrob.Agents Chemother.*, **1994**, *38*, 1573–1579.

SAMPLE

Matrix: intestinal mucosal homogenate
Sample preparation: 400 μL Homogenate mixture + 400 μL 250 mM NaCN, mix, centrifuge at 4° at 34000 g for 10 min, filter (0.45 μm) the supernatant, inject an aliquot of the filtrate.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18S (Supelco)
Column: 250 × 4.6 Suplecosil LC-18S
Mobile phase: MeOH:100 mM potassium phosphate 20:80 or MeOH:33 mM potassium phosphate 6.7:93.3
Flow rate: 1
Detector: UV 254

KEY WORDS

rat

REFERENCE

Sinko,P.J.; Hu,P. Determining intestinal metabolism and permeability for several compounds in rats. Implications on regional bioavailability in humans, *Pharm.Res.*, **1996**, *13*, 108–113.

SAMPLE

Matrix: perfusate
Sample preparation: Dilute perfusate with an equal volume of 15 mM pH 8 HEPES buffer, centrifuge at 2000 g for 2 min, inject an aliquot of the supernatant.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18S (Supelco)
Column: 250 × 4.6 Supelcosil LC-18S
Mobile phase: MeOH:100 mM potassium phosphate 20:80
Flow rate: 1
Detector: UV 254

KEY WORDS

rat; rabbit; pharmacokinetics

REFERENCE

Sinko,P.J.; Hu,P.; Waclawski,A.P.; Patel,N.R. Oral absorption of anti-AIDS nucleoside analogues. 1. Intestinal transport of didanosine in rat and rabbit preparations, *J.Pharm.Sci.*, **1995**, *84*, 959–965.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 220 × 4.6 5μm Brownlee C18
Mobile phase: MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 15:85, pH adjusted to 4.0 with 85% phosphoric acid (A) or MeCN:MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 3:15:85, pH adjusted to 4.0 with 85% phosphoric acid (B)
Column temperature: -10
Flow rate: 0.7
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 22 (A), 15 (B)

OTHER SUBSTANCES
Simultaneous: stavudine

REFERENCE
Stancato,F.A.; Srinivas,N.R.; Knupp,C.A. Effect of temperature on the high-performance liquid chromatographic separation of the anti-HIV agents, didanosine and stavudine, *Biomed.Chromatogr.*, **1996**, *10*, 29–31.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 10:90
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.85

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type anti-HIV drugs. 1. Stability studies for hairless rat skin permeation, *J.Pharm.Sci.*, **1995**, *84*, 1061–1066.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC18 DB
Mobile phase: MeCN:10 mM ammonium acetate 4:96
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.5

OTHER SUBSTANCES
Simultaneous: impurities, adenosine, deoxyinosine, dideoxyadenosine, hypoxanthine, inosine

REFERENCE
Muller,M.C.; Caude,M.; Dauphin,J.F.; Lecointre,L.; Saint-Germain,J. Use of high speed liquid chromatography (HSLC) in the pharmaceutical industry. Practical aspects and limitations, *Chromatographia*, **1995**, *40*, 394–398.

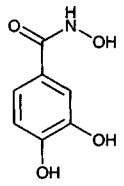
SAMPLE
Matrix: solutions
Sample preparation: Inject a 15 μL aliquot.

HPLC VARIABLES
Column: 200 × 4.6 5 μm HP Hypersil ODS
Mobile phase: MeCN:20 mM pH 7.0 Na_2HPO_4 10:90
Column temperature: 37
Flow rate: 1
Injection volume: 15
Detector: UV 265

CHROMATOGRAM
Retention time: 3.85

REFERENCE
Kim,D.-D.; Chien,Y.W. Transdermal delivery of dideoxynucleoside-type Anti-HIV drugs. 2. The effect of vehicle and enhancer on skin permeation, *J.Pharm.Sci.*, **1996**, *85*, 214–219.

Didox



Molecular formula: $C_7H_7NO_4$
Molecular weight: 169.13
CAS Registry No.: 69839-83-4

SAMPLE

Matrix: bulk
Sample preparation: Prepare a 0.1 mM solution in 10 mM KH_2PO_4, adjust pH to 6 with a few drops 5 M KOH or phosphoric acid, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 150 × 4.6 3 μm Supelcosil LC18
Mobile phase: MeOH:buffer 5:95 (Buffer was 0.05% triethylamine adjusted to pH 6 with 50 mM phosphoric acid.)
Flow rate: 0.5
Injection volume: 20
Detector: UV 255

OTHER SUBSTANCES

Simultaneous: amidox, trimidox

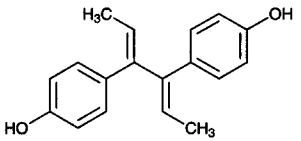
KEY WORDS

comparison with DC polarography and UV spectrophotometry

REFERENCE

Romanova,D.; Vachalkova,A.; Szekeres,T.; Elford,H.L.; Novotny,L. The new inhibitors of ribonucleotide reductase -comparison of some physico-chemical properties, *J.Pharm.Biomed.Anal.*, **1997**, *15*, 951–956.

Dienestrol



Molecular formula: $C_{18}H_{18}O_2$
Molecular weight: 226.36
CAS Registry No.: 84-17-3, 13029-44-2 (E,E)
Merck Index: 3153
Lednicer No.: 1 102

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 4.70

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM

Retention time: 6

OTHER SUBSTANCES

Simultaneous: diethylstilbestrol, trenbolone, nandrolone, zeranol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE

Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE

Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES

Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

CHROMATOGRAM

Retention time: 7 (α), 10 (β)
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: trans-diethylstilbestrol, meso-hexestrol

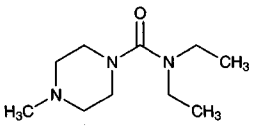
KEY WORDS

cow

REFERENCE

Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

Diethylcarbamazine



Molecular formula: $C_{10}H_{21}N_3O$
Molecular weight: 199.30
CAS Registry No.: 90-89-1, 1642-54-2 (citrate)
Merck Index: 3165
Lednicer No.: 1 278

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 2.1

OTHER SUBSTANCES

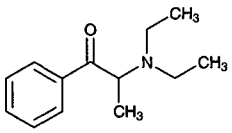
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protripty-

line, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Diethylpropion



Molecular formula: $C_{13}H_{19}NO$
Molecular weight: 205.30
CAS Registry No.: 90-84-6, 134-80-5 (HCl)
Merck Index: 3175

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 8.688

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 1 mg/mL, inject a 20 µL aliquot.

HPLC VARIABLES

Column: 250 × 5 Spherisorb S5W
Mobile phase: MeOH:buffer 90:10 (Buffer was 94 mL 35% ammonia and 21.5 mL 70% nitric acid in 884 mL water, adjust the pH to 10.1 with ammonia.)
Flow rate: 2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: 1.53

OTHER SUBSTANCES
Simultaneous: phendimetrazine, methylphenidate, phenelzine, epinephrine, pipradol, phenylpropanolamine, fencamfamin, chlorphentermine, norpseudoephedrine, phentermine, fenfluramine, methylenedioxyamphetamine, amphetamine, normetanephrine, 4-hydroxyamphetamine, bromo-STP, STP, prolintane, 2-phenethylamine, tyramine, trimethoxyamphetamine, phenylephrine, pseudoephedrine, ephedrine, methylephedrine, dimethylamphetamine, methamphetamine, mescaline, mephentermine, norpipanone, levallorphan, hydroxypethidine, normethadone, meperidine, dipipanone, diamorphine, pentazocine, acetylcodeine, monoacetylmorphine, thebacon, oxycodone, thebaine, norlevorphanol, methadone, benzylmorphine, ethylmorphine, morphine-N-oxide, codeine, codeine-N-oxide, morphine, ethoheptazine, morphine-3-glucuronide, pholcodeine, norpethidine, hydrocodone, dihydrocodeine, dihydromorphine, levorphanol, norcodeine, normorphine

Noninterfering: dopamine, levodopa, methyldopa, methyldopate, norepinephrine

Interfering: pemoline, benzphetamine, mazindol, tranylcypromine, caffeine, fenethyline, buprenorphine, dextromoramide, phenoperidine, fentanyl, etorphine, piritramide, noscapine, papaverine, naloxone, dextropropoxyphene, nalorphine, phenazocine

REFERENCE
Law,B.; Gill,R.; Moffat,A.C. High-performance liquid chromatography retention data for 84 basic drugs of forensic interest on a silica column using an aqueous methanol eluent, *J.Chromatogr.*, **1984**, *301*, 165–172.

SAMPLE
Matrix: solutions

Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica

Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7

Flow rate: 2

Injection volume: 20

Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.4

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin,

laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 2.1 Spheri-5 RP-8
Column: 220 × 2.1 Spheri-5 RP-8
Mobile phase: Gradient. A was 0.08% diethylamine and 0.09% phosphoric acid in water, pH 2.3. B was MeCN:water 90:10 containing 0.08% diethylamine and 0.09% phosphoric acid. A:B 95:5 for 2 min, to 0:100 over 15 min (?), maintain at 0:100 for 5 min.
Column temperature: 50
Flow rate: 0.5
Detector: UV 200

CHROMATOGRAM

Retention time: 9

OTHER SUBSTANCES

Simultaneous: phenylpropanolamine, ephedrine, amphetamine, methamphetamine, phentermine, fenfluramine
Also analyzed: amitriptyline, chlordiazepoxide, chlorpromazine, desalkylflurazepam, desipramine, desmethyldoxepin, diazepam, doxepin, flurazepam, imipramine, mesoridazine, norchlordiazepoxide, nordiazepam, nortriptyline, oxazepam, prazepam, promazine, thioridazine, thiothixene, trifluoperazine

REFERENCE

Rainin Catalog, C1-94, **1994**, p. 7.24.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX

Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.

Column temperature: 30

Flow rate: 2

Detector: UV 210

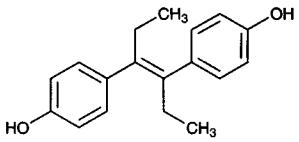
OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Diethylstilbestrol



Molecular formula: $C_{18}H_{20}O_2$
Molecular weight: 268.36
CAS Registry No.: 56-53-1
Merck Index: 3177
Lednicer No.: 1 101

SAMPLE

Matrix: blood
Sample preparation: 500 μL Plasma + 5 mL MTBE, vortex for 20 s, centrifuge at 170 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and put it into a clean tube (twice), evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL MeCN:water 45:55, inject a 50 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 3.2 5 μm Hypersil C8
Column: 150 × 3.2 5 μm Hypersil C8
Mobile phase: MeOH:30 mM pH 6 potassium phosphate buffer 58:42 (After 25 min wash column with MeOH:water 95:5 for 7 min, re-equilibrate for 13 min.)
Flow rate: 0.4
Injection volume: 50
Detector: UV 227

CHROMATOGRAM

Retention time: 17
Internal standard: diethylstilbestrol

OTHER SUBSTANCES

Extracted: paclitaxel

KEY WORDS

plasma; diethylstilbestrol is IS

REFERENCE

Sonnichsen,D.S.; Liu,Q.; Schuetz,E.G.; Schuetz,J.D.; Pappo,A.; Relling,M.V. Variability of human cytochrome P450 paclitaxel metabolism, *J.Pharmacol.Exp.Ther.*, **1995**, *275*, 566–575.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 20.882

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: Pellets. Grind 3 from each lot and extract with 10 mL MeOH with vigorous shaking for 20 min. Take a 100 μL aliquot and dilute it with 1 mL 5 μg/mL dexamethasone in MeOH. Inject a 10 μL aliquot. Oils. Dilute 1 mL in 10 mL of diethyl ether and pass 1 mL through a 5 cm Sephadex LH-20 column. Elute the drug with 8 mL dichloromethane:MeCN 94:6. Discard the first 3 mL and evaporate the remaining 5 mL under nitrogen. Dissolve the residue in 1 mL MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 1.0
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 5.4 (trans), 8.3 (cis)
Internal standard: dexamethasone (2.9)
Limit of detection: 200 ng/mL
Limit of quantitation: 400 ng/mL

OTHER SUBSTANCES
Extracted: hexestrol

KEY WORDS
comparison with TLC; pellets; oils

REFERENCE
Nascimento,E.S.; Salvadori,M.C.; Ribeiro-Neto,L.M. Determination of synthetic estrogens in illegal veterinary formulations by HPTLC and HPLC, *J.Chromatogr.Sci.*, **1996**, *34*, 330–333.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 4.68

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Injection volume: 250
Detector: UV

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: trenbolone, nandrolone, zeranol, dienestrol, hexestrol, 17α-methyltestosterone, medroxyprogesterone

REFERENCE
Jansen,E.H.J.M.; Both-Miedema,R.; van den Berg,R.H. Application of optimization procedures for the separation of anabolic compounds by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 57–64.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine,

cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: tissue

Sample preparation: Homogenize 2.5 g tissue with 10 mL acetone for 20 s, sonicate for 5 min, centrifuge at 3200 rpm. Decant the supernatant into a silanized tube. Add 8 mL acetone to the pellet and repeat the extraction. Combine the supernatants. Add to a 5 mL pipette tip containing 1.5 g alumina (80-200 mesh, Brockman activity 1) followed by an Econo-Column filled with 1.0 g AGMP-1 resin (Bio-Rad), allow to pass through by gravity. Wash with four 1 mL portions of acetone:water 95:5. Remove the alumina column, wash the ion-exchange column with 1 mL acetone:water 95:5, elute with four 1 mL portions of 10% acetic acid in acetone. Evaporate the combined eluates to dryness with nitrogen at 40°. Add 500 μL water to the residue, extract twice with 2 mL potions of ether. Combine the ether layers and evaporate them to dryness. Reconstitute the residue in mobile phase B. Inject a 20 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelco silica

Mobile phase: Gradient. A was hexane. B was MeOH:hexane:2-propanol 45:40:15. A:B from 100:0 to 60:40 over 15 min.
Flow rate: 2.0
Injection volume: 20
Detector: UV 280

CHROMATOGRAM
Retention time: 10.81
Limit of detection: 10 ng

OTHER SUBSTANCES
Extracted: estradiol, zeralenone, zeranol
Simultaneous: estrone, zeralenol, zeralanone

KEY WORDS
chicken; muscle; normal phase; SPE

REFERENCE
Medina,M.B.; Sherman,J.T. High performance liquid chromatographic separation of anabolic oestrogens and ultraviolet detection of 17β-oestradiol, zeranol, diethylstilboestrol or zearalenone in avian muscle tissue extracts, *Food Addit.Contam.*, **1986**, *3*, 263–272.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH:water 85:15, collect all eluates and pass onto Amberlite column, wash with 1 mL MeOH, 1 mL 1 M HCl, aspirate with vacuum for 1 min, elute with 2 mL 30 mM HCl in MeCN:MeOH 20:80. Evaporate eluate to dryness with nitrogen at 40°, take up in 100 μL mobile phase, inject 50 μL aliquot

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: MeCN:10 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid 48:52
Flow rate: 1.2
Injection volume: 50
Detector: E, Coulochem 5100A, detector 1 0.05 V, detector 2 0.30 V

CHROMATOGRAM
Retention time: 11
Limit of detection: 1 ng/g

KEY WORDS
muscle; liver; chicken; ox

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: tissue
Sample preparation: Homogenize (Waring blender) tissue at full speed for 2 min, lyophilize, grind. Extract with supercritical carbon dioxide at 60° at 400 atmospheres with a 20 cm × 21 μm restrictor for 1 h, collect the extract in 1 mL MeOH cooled to 5°. Evaporate the MeOH to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeCN:MeOH:20 mM ammonium formate 15:15:70, inject an aliquot. Alternatively, vortex 5 g ground tissue with 10 mL 40 mM sodium acetate, adjust pH to 4.2-4.7 with glacial acetic acid, add 100 μL β-glucuronidase (Sigma), heat at 37° for 8 h, add 20 mL MeCN, vortex for 30 s, centrifuge at 5000 rpm for 20 min. Remove a 30 mL aliquot of the supernatant and add it to 8 mL hexane and 2 mL dichloromethane, rotate for 3 min, centrifuge at 2000 rpm for 2 min. Remove a 15 mL aliquot of the middle layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 1 mL dichloromethane, inject an aliquot.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil
Mobile phase: Gradient. MeCN:MeOH:20 mM ammonium formate from 2.5:2.5:95 to 47.5:47.5:5 over 19 min.
Flow rate: 1
Injection volume: 20
Detector: UV 245 or MS, Sciex TAGA 6000E tandem triple quadrupole, APCI

CHROMATOGRAM
Retention time: 13
Limit of detection: 100 ppb

OTHER SUBSTANCES
Extracted: dexamethasone, medroxyprogesterone, melengestrol acetate, trenbolone, triamcinolone acetonide, zeranol

KEY WORDS
cow; muscle; liver; SFE

REFERENCE
Huopalahti,R.P.; Henion,J.D. Application of supercritical fluid extraction and high performance liquid chromatography/mass spectrometry for the determination of some anabolic agents directly from bovine tissue samples, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 69–87.

SAMPLE
Matrix: urine
Sample preparation: 10 mL Urine + glucuronidase/sulfatase (Helix pomatia), incubate at 37° for 1 h, extract twice with 5 mL diethyl ether, add 225 μL water and evaporate ether under nitrogen, add 400 μL MeOH, inject a 250 μL aliquot of this mixture.

HPLC VARIABLES
Guard column: 75 × 2.1 Corasil C18
Column: 150 × 4.6 5 μm Hypersil ODS
Mobile phase: MeOH:water 60:40
Flow rate: 2
Injection volume: 250
Detector: UV 240

CHROMATOGRAM
Retention time: 7.5
Limit of detection: about 6 ng/mL

OTHER SUBSTANCES

Simultaneous: 17α-methyltestosterone, 17β-trenbolone, zeranol, medroxyprogesterone, nandrolone

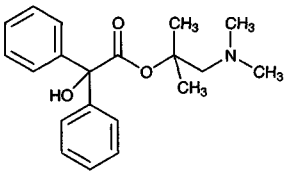
KEY WORDS

cow

REFERENCE

Jansen,E.H.; Both-Miedema,R.; van Blitterswijk,H.; Stephany,R.W. Separation and purification of several anabolics present in bovine urine by isocratic high-performance liquid chromatography, *J.Chromatogr.*, **1984**, *299*, 450–455.

Difemerine



Molecular formula: $C_{20}H_{25}NO_3$
Molecular weight: 327.42
CAS Registry No.: 80387-96-8, 70280-88-5 (HCl)
Merck Index: 3180

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM

Retention time: 13.222

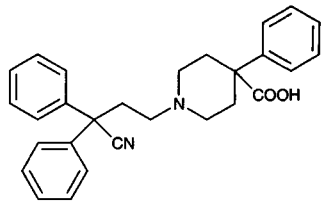
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Difenoxin



Molecular formula: $C_{28}H_{28}N_2O_2$
Molecular weight: 424.54
CAS Registry No.: 28782-42-5
Merck Index: 3183
Lednicer No.: 2 331

SAMPLE

Matrix: blood
Sample preparation: 900 μL Plasma + 100 μL mobile phase, add to a C18 Sep Pak SPE cartridge at 1 mL/min, wash with 4 mL MeCN:buffer 10:90 at 1 mL/min, elute with 4 mL MeCN:buffer 40:60 at 5 mL/min, inject a 100 μL aliquot of the eluate. (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)

HPLC VARIABLES

Guard column: C18
Column: 150 × 4.6 30 μm Ultracarb ODS (Phenomenex)
Mobile phase: MeCN:buffer 25:75 (Buffer was 0.08% diethylamine in water adjusted to pH 2.3 with orthophosphoric acid.)
Column temperature: 28
Flow rate: 1.5
Injection volume: 100
Detector: UV 210

CHROMATOGRAM

Retention time: 32
Internal standard: difenoxin

OTHER SUBSTANCES

Extracted: methadone

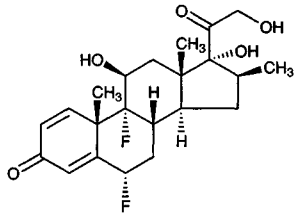
KEY WORDS

plasma; SPE; rat; difenoxin is IS

REFERENCE

Pierce,T.L.; Murray,A.G.W.; Hope,W. Determination of methadone and its metabolites by high performance liquid chromatography following solid-phase extraction in rat plasma, *J.Chromatogr.Sci.*, **1992**, *30*, 443–447.

Diflorasone



Molecular formula: $C_{22}H_{28}F_2O_5$
Molecular weight: 410.46
CAS Registry No.: 2557-49-5, 33564-31-7 (diacetate)
Merck Index: 3186

SAMPLE

Matrix: bulk, formulations
Sample preparation: Cream. Weigh out cream containing 1 mg diflorasone diacetate, add 30 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Ointment. Weigh out ointment containing 0.5 mg diflorasone diacetate, add 15 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, shake for 30 min, centrifuge at 2000 rpm for 15 min, inject a 10 μL aliquot of the lower chloroform layer. Bulk. Dissolve 1.5 mg bulk drug in 50 mL 40 μg/mL isoflupredone acetate in water-saturated chloroform, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 100 × 4.6 3 μm silica gel (Perkin-Elmer part 0258-1500)
Mobile phase: Butyl chloride:dichloromethane:THF:acetic acid 70:25:2:3 (Butyl chloride and dichloromethane were saturated with water.)
Flow rate: 2.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 11 (diflorasone diacetate)
Internal standard: isoflupredone acetate (24)

OTHER SUBSTANCES

Simultaneous: related compounds

KEY WORDS

cream; ointment; normal phase

REFERENCE

Shaw,M.C.; Vanderwielen,A.J. Liquid chromatographic assay for diflorasone diacetate in cream and ointment formulations, *J.Pharm.Sci.*, **1984**, *73*, 1606–1608.